Photochemical depolymerisation of dermatan sulfate and analysis of the generated oligosaccharides

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Abstract

Radical depolymerisation is the method of choice for the depolymerisation of glycosaminoglycans (GAGs), especially when enzymatic depolymerisation cannot be performed due to the lack of suitable enzymes. The established Fenton type free radical depolymerisation generates radicals from a solution of H_2O_2 in the presence of Cu^{2+} or Fe^{2+}. When applied to dermatan sulfate (DS), the Fenton type depolymerisation of DS (Panagos, Thomson, Bavington & Uhrin, 2012)produced exclusively oligosaccharides with reducing end GalNAc , which was partially oxidized to acetylgalactosaminic acid. We report here the results of the TiO_2 catalyzed photochemical depolymerisation of DS. NMR analysis of these DS oligosaccharides revealed the presence of reducing end IdoA, observed for the first time. The reducing end acetylgalactosaminic acid was also detected. The photochemical depolymerisation method thus enables preparation of new types of GAG oligosaccharides suitable for further biochemical and biological investigation.

Highlights

- Free radical photochemical depolymerisation generates GAG oligosaccharides.
- The structures of DS oligosaccharides were studied by NMR.
- Oligosaccharides with uronic acid as the reducing end monosaccharides were found.
- Acetylgalactosaminic reducing end monosaccharides were also generated.
- No desulfation of the generated oligosaccharides was found.

Keywords

NMR; glycosaminoglycans; dermatan sulfate; photochemical depolymerisation.

Chemical compounds studied in this article

Phosphate-buffered saline (PubChem CID: 24978514); Titanium dioxide (PubChem CID: 26042), Ethylenediaminetetraacetic acid (PubChem CID: 6049), Trimethylsilyl propionate (PubChem CID: 519321), Dermatan sulfate (Pubchem CID:32756).

Abbreviations

GAGs, glycosaminoglycans; LMWH, low molecular weight heparin; DS, dermatan sulfate; GalNAc, N-acetylgalactosamine; IdoA, iduronic Acid; HSQC, Heteronuclear single quantum coherence; TOCSY, Total correlation spectroscopy; HMBC, Heteronuclear multiple bond coherence;

1. Introduction

Glycosaminoglycans (GAGs) are a biologically important class of carbohydrates usually found on cell surfaces in the form of proteoglycans (Dietrich, Sampaio, Montesdeoca & Nader, 1980; Mathews, 1975; Medeiros, Mendes, Castro, Baú, Nader & Dietrich, 2000). They are essential for a variety of cell-cell signaling events, such as cell growth and cell adhesion (Singer, 1992). As long unbranched polysaccharides, GAGs also participate in the construction of connective tissues (Comper & Laurent, 1978; Mathews, 1975), such as cartilage. The ability of GAGs to be involved in such a variety of biochemical processes is underpinned by their inherent structural heterogeneity.

GAGs are built from repeating disaccharide units, which typically consist of an uronic acid (iduronic acid or glucuronic acid) and a hexosamine (galactosamine or glucosamine). Furthermore, these disaccharide units can be sulfated at different sites and the position and type of the glycosidic linkage also varies between GAG types. These attributes lead to structural variety within the GAG family. Although not random, the epimerization and sulfation events are not regular, creating large heterogeneity within a single GAG type. For example, during the processing of heparan, the precursor of heparan sulfate (HS), various enzymes including N-deacetylases/N-sulfotransferases, as well as 6-O- and 3-O sulfotransferases and C-5 epimerases, modify its structure, though these modifications are neither random or complete (Vreys & David, 2007). The resulting macroscopic organization of HS shows a typical ‘block structure’ containing extensive structural heterogeneity (Gallagher, 2006).

In order to study the biology and pharmacology of these heterogeneous polysaccharides it is necessary to identify specific structures within their chains, which are responsible for their distinct biological properties (Toida, Sato, Sakamoto, Sakai, Hosoyama & Linhardt, 2009). For example, the study of heparin oligosaccharides revealed the existence of a specific pentasaccharide sequence responsible for the binding of heparin to antithrombin (Munoz & Linhardt, 2004). GAG oligosaccharides are also important in their own right, e.g. low molecular weight heparin (LMWH) (Choay, Lormeau, Petitou, Sinaÿ & Fareed, 1981), has almost completely replaced unfractionated heparin as a pharmaceutical (Hileman, Smith, Toida & Linhardt, 1997). Low molecular weight dermatan sulfates (DS) also show increased bioavailability without the loss of bioactivity (Dol et al., 1990; Legnani et al., 1994).

Smaller GAG oligosaccharides can lead to a reduction in the adverse effects sometimes associated with full-length polysaccharides. For example, LMWH decreases the occurrence of heparin induced thrombocytopenia due to less efficient interaction with platelet factor 4 (Gray, Mulloy & Barrowcliffel, 2008). LMWH also reduces the risk of osteoporosis, another side effect of heparin (Matzsch, Bergqvist, Hedner, Nilsson & Ostergaard, 1990; Monreal, Vinas, Monreal, Lavin, Lafoz & Angles, 1990). Another example is the depolymerisation of fucosylated chondroitin sulfate, fCS, from sea cucumber (Panagos et al., 2014; Suzuki, Kitazato, Takamatsu & Saito, 1991; Wu, Xua, Zhao, Kang & Ding, 2010; Wu et al., 2013; Yang, Wang, Jiang, Lv, Zhang & Lv, 2015), which is critical for antithrombotic applications, due to an undesired platelet aggregation associated with long polysaccharide chains (Wu, Xu, Zhao, Kang & Ding, 2010b).

Depolymerisation of GAGs and the production of fully characterised oligosaccharides is therefore an essential process in evaluation of the biological potential of these molecules. A variety of different depolymerisation methods, characterized by specific advantages and limitations, have therefore been developed to date. The most widely used is enzymatic depolymerisation, although this method also has its limitations. For example, highly sulfated GAGs, which are typical for marine species, are resistant to enzymatic depolymerisation (Vieira, Mulloy & Mourao, 1991). Moreover, oligosaccharides generated by heparanases and chondroitinases contain an unsaturated uronate residue as the non-reducing end monosaccharide and a hexosamine as the reducing end monosaccharide (Pervin, Gallo, Jandik, Han & Linhardt, 1995). Finally, depolymerisation enzymes can be very expensive, e.g. one mg of chondroitinase ABC lyase, which can deliver between 50 to 250 units, can cost much more than £ 1000.

Other depolymerisation methods include acid hydrolysis, solvolysis and ultrasonication. Acid hydrolysis is a chemically based approach which involves both the depolymerisation and the desulfation of the polysaccharide (Mourao et al., 1996) and for that reason it is not widely used on sulfated GAGs. Solvolytic depolymerisation has also been shown to be effective on GAGs but it also involves desulfonation of the polysaccharide chains and is also known to structurally modify the oligosaccharide products (Toida, Sato, Sakamoto, Sakai, Hosoyama & Linhardt, 2009). Ultrasonic depolymerisation is unique among the depolymerisation methods, as it is a mechanical method, which has been employed for the depolymerisation of hyaluronic acid (Miyazaki, Yomota & Okada, 2001). Although this technique generates lower molecular weight GAGs with unmodified chains, its application is limited because of the size of produced species (MW>10^5 Da), which are much bigger than those generated by other methods.

One of the more widely used chemical depolymerisation methods suitable for GAGs is the free radical Fenton-type depolymerisation. It has been used not only to generate oligosaccharides from heparin (Nagasawa, Uchiyama, Sato & Hatano, 1992), chondroitin and dermatan sulfate (Ofman, Slim, Watt & Yorke, 1997), and hyaluronate (Uchiyama, Dobashi, Ohkouchi & Nagasawa, 1990), but also marine GAGs (Wu, Xu, Zhao, Kang & Ding, 2010a). However, Fenton-type depolymerisation reaction is known to preferentially degrade unsulfated IdoA in heparin (Nagasawa, Uchiyama, Sato & Hatano, 1992) and DS (Ofman, Slim, Watt & Yorke, 1997; Panagos, Thomson, Bavington & Uhrin, 2012), thus creating oligosaccharides that have hexosamine at the reducing end, similar to enzymatic depolymerisation. In addition, this reducing end hexosamine can sometimes be oxidized, as seen in heparin and DS, forming acetylglucosaminic acid and acetylgalactosaminic acid, respectively (Panagos, Thomson, Bavington & Uhrin, 2012; Vismara, Pierini, Guglieri, Liverani, Mascellani & Torri, 2007; Vismara et al., 2010).

In this work we present a structural analysis of oligosaccharides obtained by a photochemical depolymerisation of a model GAG, DS, and compare them with DS oligosaccharides obtained previously by the Fenton-type depolymerisation (Panagos, Thomson, Bavington & Uhrin, 2012), as both methods use free radicals to cleave the glycosidic bonds. The photochemical depolymerisation has been used recently to depolymerise alginate (Burana-osot, Hosoyama, Nagamoto, Suzuki, Linhardt & Toida, 2009), pectin (Burana-osot, Soonthornchareonnon, Hosoyama, Linhardt & Toida, 2010), K5 heparosan (Higashi et al., 2011) and heparin (Higashi et al., 2012). While the Fenton type depolymerisation generates free radicals from hydrogen peroxide using an iron or copper catalyst, photochemical depolymerisation uses UV light and titanium dioxide to generate radicals in an aqueous environment, making it a very inexpensive technique.

2. Materials and methods

2.1 Materials

Titanium(IV) oxide (TiO_2) anatase powder was purchased from Acros Organics. Porcine DS, containing more than 95% of the repeating disaccharide [-β-D-GalNAc4S-(1 → 4)-α-L-IdoA-(1 → 3)]$_n$ was obtained from Celsus Laboratories. Ethylenediaminetetraacetic acid (EDTA) and trimethylsilyl propionate (TSP) were purchased from Goss Scientific Instruments Ltd. and Aldrich, respectively.

2.2 Depolymerisation of polysaccharides by photochemical reaction

Samples (300 mg) were dissolved in 30 ml of deionised water in a shallow open crystallising dish to a final concentration of 10 mg/ml. 30 mg (1:10) of titanium(IV) oxide (TiO_2) anatase powder (Acros Organics) was added. A magnetic stirrer was added to the sample to ensure the dissolution of atmospheric oxygen in the solution and to prevent settling of TiO_2. The receptacle was placed under a UV light source (125 W low pressure mercury lamp from Photochemical Reactors Ltd). In order to maximise UV absorbance by the TiO_2 the lamp was placed 10 cm from the receptacle at approximately a 70° angle. The reaction was carried out at room temperature and was stopped after 34 h, when the reaction progress was deemed to be satisfactory. 100 µl samples were taken at regular intervals (8-10 hours) and the progress of the reaction was monitored by HPLC, following the method described below. The samples were centrifuged at 16,100 × g for 5 minutes at room temperature. The supernatant was passed through a 0.22 µm membrane filter for complete removal of the TiO_2 particles. The samples were separated by size exclusion chromatography (SEC) at room temperature for 45 minutes at a flow rate of 0.5 ml/min. A Superdex Peptide 10/300 GL size exclusion column attached to a Waters 600 LCD HPLC equipped with a 486 Tunable Absorbance Detector was deployed. PBS (Phosphate Buffered Saline tablets, Fisher Scientific) was used as mobile phase and the UV absorbance was recorded at 218 nm, as the absence of a double bond generated from enzymatic depolymerisation, prevents the use of the absorbance at 232nm. The total reaction mixture after the end of the reaction was centrifuged for 30 min at 2,742 × g and the supernatant was passed through a 0.22 µm membrane and freeze-dried.

2.3 Fractionation and isolation of the oligosaccharides

The freeze-dried material obtained from the depolymerisation reaction was redissolved in 2.5 ml deionised water and fractionated by SECon a BioCAD 700E Workstation FPLC system equipped with a Waters Fraction Collector. Two XK26/100 columns preceded by a XK26/20 guard column (GE healthcare) were packed with Bio-Gel P-10 Fine resin (Bio-Rad Laboratories) and connected in series. Enzymatically cleaved DS tetrasaccharides and hexasaccharides were used as standards. The sample was loaded on a 5 ml loop and was run at a flow rate of 0.4 ml/min for a total of 62.5 h, with PBS as the mobile phase. The UV absorbance was recorded at 218 nm and the fractions containing oligosaccharides were collected, pooled and freeze dried.

Prior to NMR experiments, the lyophilized samples were desalted using the BioCad 700E system equipped with a XK16/40 column packed with Sephadex G25 superfine (Sigma-Aldrich). Each run was carried out with an injection volume of 5 ml and dH_2O as mobile phase. For each run, the injection loop was first flushed for 6 minutes at 2.5 ml/min and then the flow rate was increased to 4 ml/min for 21 minutes and 3 ml fractions were collected. The UV absorbance was recorded at 218 nm. Four to five fractions were pooled together, yielding desalted oligosaccharides in 12-15 ml of solution. The samples were subsequently freeze-dried.

2.4 Structural analysis of the fractions by NMR

After lyophilisation the samples were dissolved in 99.9% D_2O, (Aldrich, 540 µl) containing deuterated NaH_2PO_4 + HNa_2HPO_4 buffer (10 mM, pH 7.2). A stock solution (20 µl) of EDTA and TSP was added. The stock solution was prepared by dissolving EDTA (4 mg) and TSP (9 mg) in the phosphate buffer (200 µl). The pH was adjusted to 7.2 by adding few drops of a concentrated solution of NaOH in D_2O. All spectra were acquired at 50 ◦C on an 800 MHz Avance I Bruker NMR spectrometer equipped with a z-gradient triple-resonance TCI cryoprobe. The spectra were referenced (0 ppm) using the ^{1}H and ^{13}C signals of TSP.

1D ^{1}H NMR spectra were acquired using relaxation and acquisition times of 1.5 and 0.4999 s, respectively; 32 scans per spectrum were accumulated. 1D ^{13}C NMR spectra with ^{1}H GARP decoupling were acquired using relaxation and acquisition times of 4 and 0.6816 s, respectively; 31504 scans per spectrum were accumulated in 41 h. The FIDs were zero filled once and a 2 Hz exponential line broadening was applied prior to Fourier transformation. 2D ^{1}H, ^{13}C HSQC spectra were acquired using a modified HSQC sequence. This pulse sequence employs DEPT editing sequence, which ensured that the CH_2 signals appear as negative peaks. This was invaluable for the identification of C6 atoms of GAGs. The polarization transfer was optimised for $^{1}J_{CH}$ = 150 Hz for all the experiments and ^{13}C adiabatic decoupling was used to decouple the carbon-proton couplings. Spectra were acquired using t_1 and t_2 acquisition times of 0.011 and 0.1069 s, respectively; 4 scans were acquired into each of 512 F_1 complex data points resulting in the total experimental times of 1.5 h per sample.

2.5 Fractionation of fucosylated chondroitin sulfate.

The samples of fCS was depolymerized and purified as described previously (Panagos et al., 2014).

3. Results

3.1 Fractionation of DS and isolation of the DS oligosaccharides

Samples taken from the reaction mixture containing DS and TiO_2 in water as described in detail in 2.2, were analysed periodically to monitor the progress of the reaction. Monitoring by HPLC showed that the photochemical depolymerisation of DS does not progress at a constant rate under the conditions used. As indicated by the chromatogram of Figure 1a, the peak eluting with the column void volume, containing oligosaccharides with M_w>7000 Da is almost intact after the first 24 h. The reaction seems to progressively accelerate and after 34 h the peak eluting with the void volume has decreased significantly. At the same time the presence of smaller oligosaccharides is clearly visible (Figure 1b). The reaction was therefore stopped at this point and the reaction mixture was separated using a SEC column. The SEC chromatogram (Figure 1c) shows separation of fragments; the fraction labelled **I**, composed from fractions 77-83 (total volume 70 ml) was chosen for structural analysis, since its elution time was similar to that of a DS tetrasaccharide standard, run previously during the column calibration (data not shown). A shoulder visible on the right hand side of peak **I** is most likely due to co-elution trisaccharide species as discussed later, while the peak eluting at approximately 1500 minutes belongs to the disaccharides fractions The peaks eluting later contained fragments that when analysed by NMR, were identified as smaller than the disaccharide, potentially monosaccharides or even smaller entities.

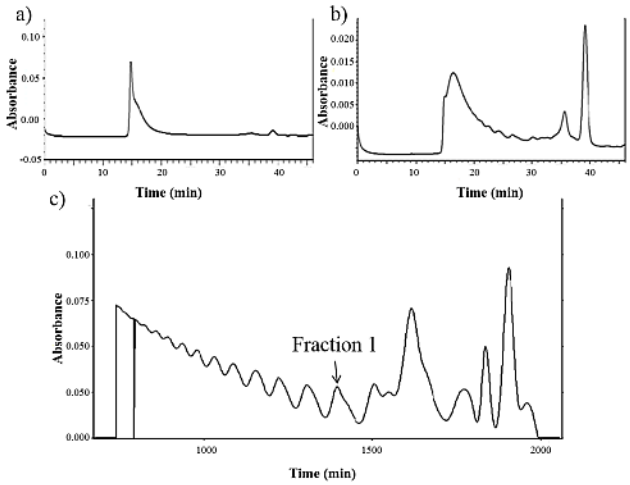

Figure 1: Chromatograms of the depolymerized DS (a) and (b) show HPLC traces after 24 and 34 hours, respectively. (c) Screenshot of the final 1500 minutes of the size exclusion chromatogram of the final reaction mixture obtained after 34 h of depolymerisation. The UV absorbance was monitored at 218 nm and the fraction brought forward for the analysis is indicated.

3.2 NMR analysis of fraction **I**

Fraction **I** was analyzed by 1D and 2D NMR methods and the structures of the constituting oligosaccharides were determined. This process was facilitated by a comparison with a previously analyzed tetrasaccharide fraction generated by the Fenton type free radical depolymerisation of DS, which used H_2O_2 to generate radicals and Cu^{2+} as the catalyst (Panagos, Thomson, Bavington & Uhrin, 2012). This also gave us the opportunity to compare the outcomes of the Fenton type and photochemical depolymerisation. It became immediately obvious that the spectra of fraction **I** contain some additional strong signals. Although the anomeric region of the ^{13}C spectrum containing the non-reducing rings signals was practically identical for samples generated by both methods, new strong signals (95.12 and 97.50 ppm) were found in the anomeric region of the reducing rings for the photochemically depolymerized fraction **I** (Figure 2).

These new strong signals belong to the reducing end α- and β-IdoA and their integral intensities have almost the same total intensity as the reducing end GalNAc signals (Figure 2a, b). For comparison, in the case of Fenton type depolymerisation the IdoA signals contained less than 10% of the total reducing end signals. This means that the photochemical depolymerisation does not proceed to fully oxidise the released reducing end IdoA, as was the case with the Fenton type depolymerisation.

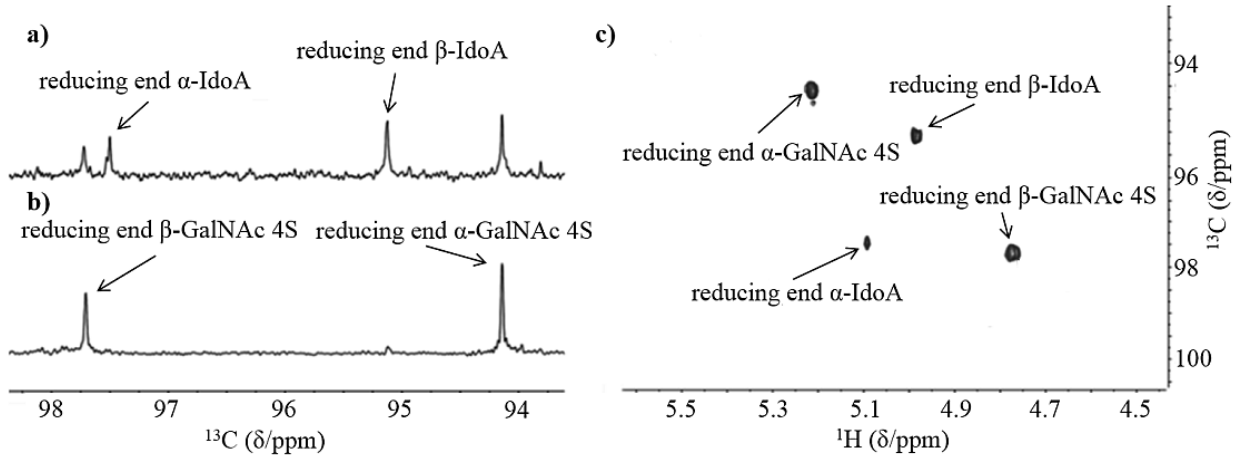

Figure 2: Comparison of the reducing end anomeric regions of NMR spectra. 1D ^{13}C spectra of tetrasaccharides obtained by (a) photochemical (fraction I) and (b) Fenton type depolymerisation. (c) A partial 2D ^{1}H, ^{13}C HSQC spectrum of fraction I tetrasaccharides obtained by photochemical depolymerisation.

It is now well established, that Fenton type depolymerisation proceeds to partially oxidise the released reducing end HexNAc as seen in heparin and DS, forming acetylglucosaminic acid and acetylgalactosaminic acid, respectively (Panagos, Thomson, Bavington & Uhrin, 2012; Vismara, Pierini, Guglieri, Liverani, Mascellani & Torri, 2007; Vismara et al., 2010) Focusing on the most prominent C2 acetylgalactosaminic acid signal at 58.23 ppm, it is evident (Figure 3) that this signal is stronger in the photochemically depolymerized sample. Here the acetylgalactosaminic acid accounts for ~22% of the total reducing end GalNAc signals, whereas in case of Fenton type depolymerisation it was less than

13%. It is notable that the analysis of the 2D ^{1}H ^{13}C HSQC spectrum of the sample showed that there were no signals corresponding to the open chain reducing end IdoA, which indicates that opening of the IdoA monosaccharide leads to its complete degradation.

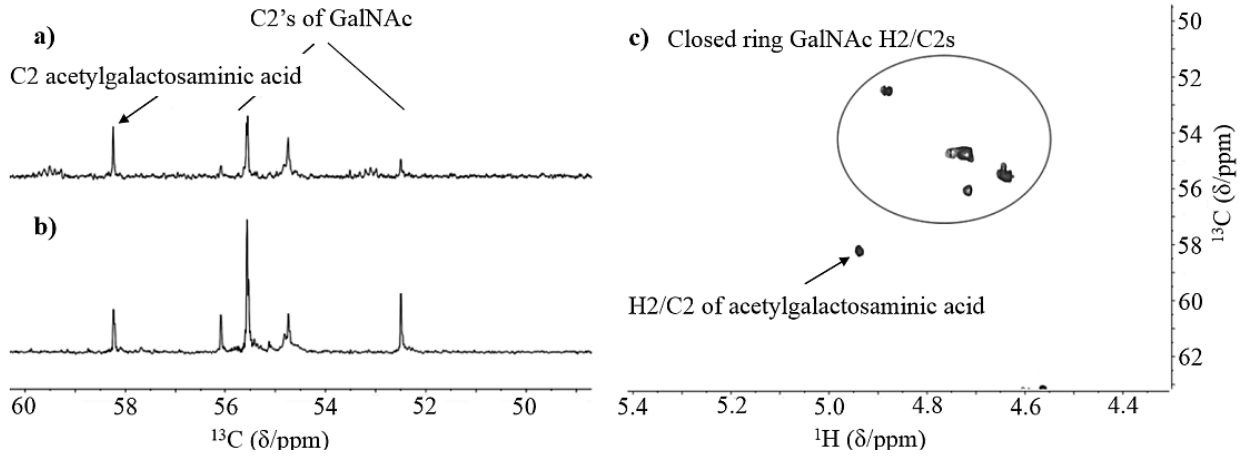

Figure 3: Comparison of the GalNAc C2 region of the NMR spectra of tetrasaccharides obtained by (a) photochemical and (b) Fenton type depolymerisation. (c) A partial 2D ^{1}H, ^{13}C HSQC spectrum of the H2/C2 region of fraction I.

Finally, a comparison of the integrals of the reducing and non-reducing anomeric ^{13}C signals of fraction I showed ratio of 1:2.7. If this fraction contained purely tetrasaccharides this should be 1:3. Since the anomeric carbon of the terminal acetylgalactosaminic acid is oxidised and does not resonate in the integrated anomeric region, this ratio should be even larger, reflecting the presence of 20% of the open chain species in fraction **I**. This apparent discrepancy can be explained by the presence of trisaccharides in fraction **I**, as additionally confirmed by the analysis of the MS data (data not shown). At the same time the IdoA and GalNAc reducing-end signals show intensity ratio of 1:1. It is therefore logical to assume that both IdoA-GalNAc-IdoA and GalNAc-IdoA-GalNAc trisaccharides are present in fraction **I**.

Photochemical depolymerisation was also performed on fucosylated chondroitin sulfate from *H. forskali* (fCS) as described previously (Panagos, Thomson, Bavington & Uhrin, 2012). The repeating trisaccharide unit of this fCS, →3)GalNAc4,6S(1β→4)[FucαX1→3]GlcA(1β→, contains branching fucose. The X in this formula signifies different sulfation patterns of fucose and stands for sulfation at positions 3,4 or 2,4 or 4, which were present at levels of 46%, 39% and 15%, respectively. Other sea cucumber species produce similar polysaccharides that differ only in the level of sulfation (Vieira & Mourao, 1988; Wu et al., 2013; Yoshida, Minami, Nemoto, Numata & Yamanaka, 1992). As this polysaccharide is resistant to enzymatic depolymerisation, it is therefore a good candidate for photochemical depolymerisation. The branched structure and heterogeneity of the sulfation predisposes the fCS polysaccharide to yield a more complex mixture of oligosaccharides than the one generated from DS, which prevented their full characterization. Nevertheless, similar features were observed as for the DS. In particular, the amount of the N-acetylgalactosaminic acid was significantly increased in smaller fractions, exceeding 60% of the total reducing end content of GalNAc (data not shown). The oligosaccharides fractions (dp3-dp10) were used in microarray binding assays and were shown to bind to L- and P-selectins (Panagos et al., 2014). Since fCS-selectin binding is dependent on the presence of sulfated fucosylated branches, these results

indicate that photochemical depolymerisation neither cleaved the fucose branches nor caused desulfation of the polysaccharide.

4. Discussion and conclusions

Catalytic photochemical depolymerisation of DS was achieved by titanium dioxide in the presence of UV light. In this study we have modified the controlled depolymerisation reaction reported previously (Burana-osot, Hosoyama, Nagamoto, Suzuki, Linhardt & Toida, 2009; Burana-osot, Soonthornchareonnon, Hosoyama, Linhardt & Toida, 2010; Higashi et al., 2012; Higashi et al., 2011), in order to achieve a larger scale (x30 times) and without the use of specialized equipment. A wide range of oligosaccharides, from dp2 to dp22 as shown by SEC, was obtained for DS. These oligosaccharides contained both odd and even numbered species with no desulfation observed.

The tetrasaccharide fractions generated by the Fenton type and photochemical depolymerisation of DS were compared (Panagos, Thomson, Bavington & Uhrin, 2012). Despite the fact that both of techniques are based on oxygen free radicals creating random scissions of mainly glycosidic linkages, important structural differences between the two tetrasaccharides were observed. These differences could be caused by possible differences in the free radicals generated through the two techniques. Fenton-type reaction depolymerisation generates almost exclusively hydroxyl radicals, which are known as the most reactive species of the oxygen radical family (Vismara, Pierini, Guglieri, Liverani, Mascellani & Torri, 2007). On the other hand excitement of TiO_2 by UV leads to the generation of superoxide radicals. At the same time, the more reactive hydroxyl radicals are produced (Xiang, Yu & Wong, 2011).

The most notable difference between the two depolymerisation methods was that the photochemical depolymerisation did not degrade unsulfated IdoA as reported for heparin (Nagasawa, Uchiyama, Sato & Hatano, 1992) and DS (Ofman, Slim, Watt & Yorke, 1997; Panagos, Thomson, Bavington & Uhrin, 2012) depolymerised by Fenton type reaction. Photochemical depolymerisation is, up to now, the only depolymerisation method that preserves reducing end unsulfated IdoA (Figure 4). This opens the possibility of creating a wider range of oligosaccharides that could have novel biological activities.

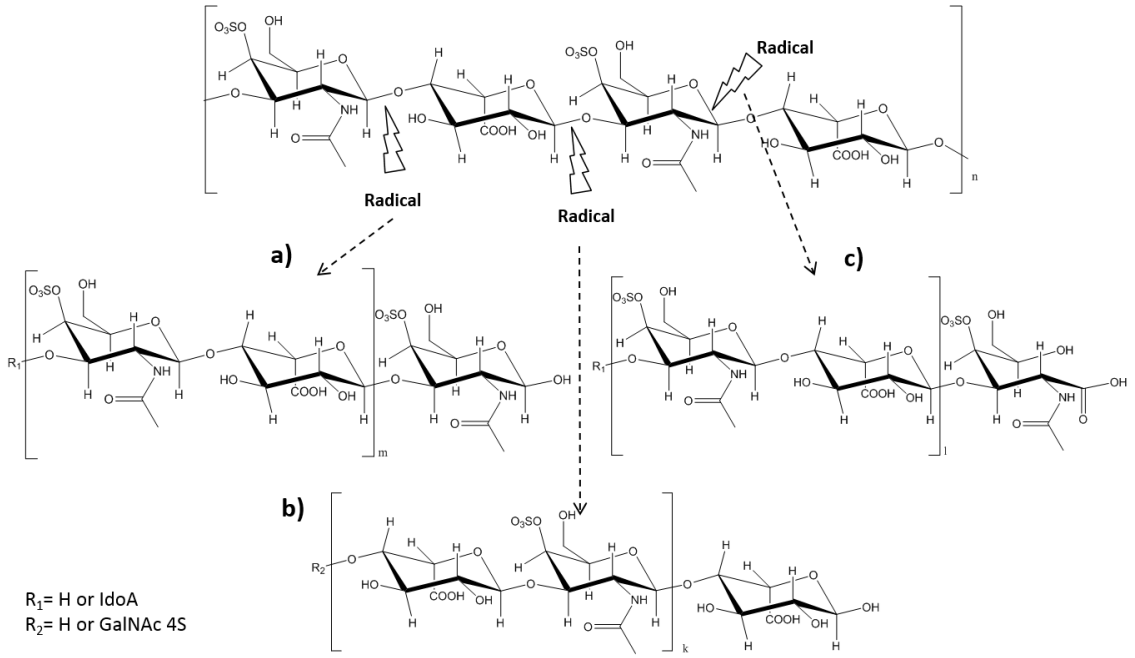

Figure 4: Free radical depolymerisation of a DS polysaccharide, produced oligosaccharides with a) reducing end GalNAc, b) reducing end IdoA and c) reducing end acetylgalactosaminic acid. The non-reducing end terminal monosaccharide can be either IdoA or GalNAc 4S, as indicated. NMR analysis showed no evidence of changes in the structure of non-reducing end monosaccharides after photochemical depolymerisation.

While the photochemical depolymerisation has a milder effect on the reducing end IdoA than the Fenton type depolymerisation, this is not the case for the reducing end GalNAc. The NMR analysis showed that the percentage of reducing end GalNAc oxidised to N-acetylgalactosaminic acid increased compared to the Fenton type depolymerisation. These differences cannot therefore be fully justified by stating that the photochemical depolymerisation is milder; they rather imply subtle differences in molecular mechanism between the two methods, possibly due to the differences in the radicals produced.

Since photochemical depolymerisation did not degrade reducing end IdoA, and assuming that it also cleaves glycosidic linkages randomly, the existence of a trisaccharide in the DS fractions was to be expected. This was verified by NMR and MS (Panagos, Thomson, Bavington & Uhrin, 2012) and DS trisaccharides (M_W 653.5 or 760.6 g/mol) were shown to be co-eluted with the tetrasaccharide (M_W 936.7 g/mol) fraction.

In summary, this study describes a larger scale photochemical depolymerisation of a simple (DS), which did not require pH monitoring or specialized equipment. This method generated a range of oligosaccharides with both odd and even numbers of sugar residues, as previously reported for heparin (Higashi et al., 2012). Additionally, structural differences were uncovered, by 1D and 2D NMR, between the oligosaccharides generated by photochemical depolymerisation and Fenton type depolymerisation. These results indicate that photochemical depolymerisation should be classified as a distinct depolymerisation technique that enables production of oligosaccharides containing unsulfated reducing-

end uronic ucid and saturated non-reducing end monosaccharides rings. The photochemical depolymerisation method thus enables preparation of new class of GAG derived oligosaccharides that now can be investigated for their biochemical/biological properties.

Acknowledgements

This work was financially supported by KTP009399 award from the Technology Strategy Board and Scottish Funding Council and GlycoMar Ltd.

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